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Masana Ogata*

Junko Mizugaki†

*Okayama University,

†Okayama University,

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The reaction between acatalasemia hemolysate and anti-normal erythrocyte catalase IgG gave a precipitin line which was interrupted by a normal precipitin line, in the double immunodiffusion method. Hypocatalasemia hemolysate revealed two precipitin lines, one completely, the other partially, fused with a normal precipitin line. No immunological reactions between fresh hemolysates of acatalasemia, hypocatalasemia and normal erythrocytes and antihuman C-reactive protein serum were observed, but the lysates stored for 4 weeks in an ice cold water bath reacted slightly with this antiserum.

KEYWORDS: heterogeneity, C-reactive protein, immunodiffusion

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IMMUNOLOGICAL PROPERTIES OF ERYTHROCYTE CATALASE IN JAPANESE TYPE ACATALASEMIA

Masana OGATA and Junko MIZUGAKI

Department of Public Health, Okayama University Medical School, Okayama 700, Japan

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Abstract. The reaction between acatalasemia hemolysate and anti-normal erythrocyte catalase IgG gave a precipitin line which was interrupted by a normal precipitin line, in the double immunodiffusion method. Hypocatalasemia hemolysate revealed two precipitin lines, one completely, the other partially, fused with a normal precipitin line. No immunological reactions between fresh hemolysates of acatalasemia, hypocatalasemia and normal erythrocytes and antihuman C-reactive protein serum were observed, but the lysates stored for 4 weeks in an ice cold water bath reacted slightly with this antiserum.

Key words : heterogeneity, C-reactive protein, immunodiffusion.

Japanese type acatalasemic homozygotes (acatalasemias) have residual catalase activity in their erythrocytes whereas heterozygotes (hypocatalasemias) have catalase activity about half the normal enzyme level in their erythrocytes. The other properties of the catalase in acatalasemia and hypocatalasemia are similar to the normal enzyme, such as stability to heat and some enzyme inhibitors, electrophoretic mobility, and immunological reaction against anti-normal human erythrocyte catalase serum (1-4). The presence of C-reactive protein (CRP) in normal and acatalasemia hemolysates and a similar antigenicity to erythrocyte catalase were reported by Nishimura, *et al.* (5). The chemical similarity of tryptic peptide maps between human CRP and human hepatic catalase was reported by Hokama, *et al.* (6).

Differences in an enzyme deficiency condition between Japanese and Swiss types of acatalasemia have been reported by Aebi, *et al.* (7, 8). Comparative analysis of the enzyme of acatalasemic homozygotes showed that Swiss type acatalasemic heterozygotes have catalase with intermediate thermolability and electrophoretic mobility. The catalase activity of heterozygotes falls within the normal activity range (9).

We have made efforts to obtain purified catalase from the Japanese type acatalasemia and from normal erythrocytes for the detection of heterogeneity of the enzyme. Preparatory artifacts could not be completely avoided during the purification process. In this report, fresh hemolysates obtained from acatalasemia, hypocatalasemia and normal erythrocytes were analysed by immunological techniques.

MATERIALS AND METHODS

Fresh blood samples with heparin as an anticoagulant were obtained from an acatalasemia and a hypocatalasemia belonging to the GIO. family, of which the genotype and phenotype of the biochemical defect are known to Ogura *et al.* (10). Packed red cells were washed three times with cold phosphate buffered saline (containing 10 mM phosphate, pH 7.0) and the buffy coat removed. One volume of red cells was hemolyzed with 1.5 volumes of distilled water, stirred, and kept for 1 h at 4°C.

Anti-normal human erythrocyte catalase IgG fraction was purchased from Miles Laboratories, Inc, USA. Antihuman CRP rabbit serum was purchased from Wako Pure Chemical Industries, Ltd, Japan and the standard human CRP serum was kindly supplied by Japan Lyophilization Laboratory, Japan. Immunodiffusion was performed in 1.2% agarose (Hoechst, Germany) gel in 0.05 M citrate buffered saline (pH 7.0) according to Nishimura *et al.* (5). Antigen wells 4 mm in diameter were punched out at a distance of 4 mm from antibody well and then filled with the hemolysates. After 48 h, the immunodiffusion plate was left in saline for 24 h and stained with 0.2% Coomassie Brilliant Blue R-250 (CBB) purchased from Nakarai Chemicals, Ltd, Japan.

RESULTS

The properties of fresh hemolysates from acatalasemia, hypocatalasemia and normal erythrocytes against anti-normal erythrocyte catalase IgG were compared by the double diffusion test. In the reaction with anti catalase IgG, the acatalasemia hemolysate formed a precipitin line closer to the antibody well than normal hemolysate. This line was interrupted by a precipitin line of normal hemolysates (Fig. 1, 1 and 2). Two precipitin lines formed in the reaction of hypocatalasemia hemolysate and antibody (Figs. 1, 2). One line was interrupted by a line of normal hemolysate and the other was completely fused with the normal line. Similar results were obtained when the mixture of acatalasemia and normal hemolysates reacted with antibody. The mixture of hypocatalasemia and normal hemolysate reacted with antibody and formed a slightly dif-

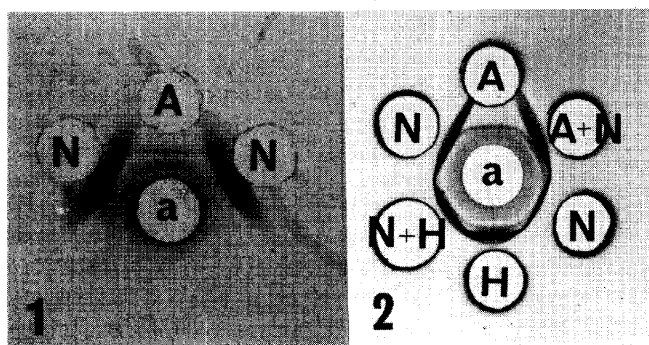


Fig. 1. Double immunodiffusion test of hemolysates obtained from acatalasemia (A), hypocatalasemia (H) and normal (N) erythrocytes using anti-normal erythrocyte catalase IgG (a). A mixture of hemolysate A and N (A + N) or of H and N (H + N) was examined at the same time.

fused line which was completely fused with the normal line.

Fresh hemolysates (*i.e.* not more than one day storage in an ice cold water bath) obtained from acatalasemia, hypocatalasemia and normal erythrocytes did not react with antihuman CRP serum. None of the sources of hemolysate reacted with this antiserum during a week of storage in ice water, but all, reacted with this antiserum after 4 weeks storage under the same conditions to form a faint precipitin line of partial fusion with that of the standard human CRP serum (Fig. 2). All the stored hemolysates reacted with anticatalase IgG in much the same way as the fresh hemolysates.

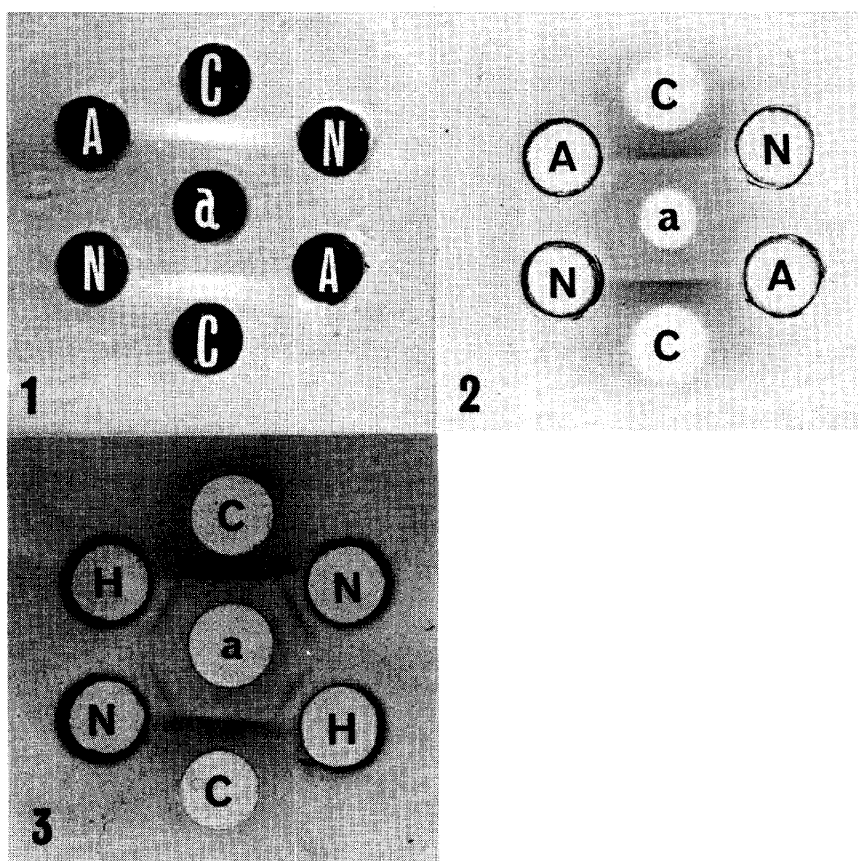


Fig. 2. Double immunodiffusion test of hemolysates obtained from acatalasemia (A), hypocatalasemia (H) and normal (N) erythrocytes using antihuman C-reactive protein serum (a). C; C-reactive protein. No reaction was observed between hemolysates prepared in a day and antiserum (1). Hemolysates stored for 4 weeks reacted with antiserum (2 and 3).

DISCUSSION

In the double immunodiffusion test, a precipitin line indicating antigenic similarity is formed between partially purified normal erythrocyte catalase, partially purified acatalasemia erythrocyte catalase, and anti-normal human erythrocyte catalase IgG (3). Moreover, there is apparently complete antigenic identity between normal and variant catalases by the double immunodiffusion method when purified enzyme preparations are used (11). In neither of these instances, however, does a precipitin line appear between acatalasemia hemolysate and anti-normal erythrocyte catalase IgG. The present experiment demonstrates heterogeneity of erythrocyte catalase in the fresh hemolysates obtained from acatalasemia, hypocatalasemia and normal individuals by the double immunodiffusion test using anti-normal erythrocyte catalase IgG.

Hemolysates obtained from acatalasemia, hypocatalasemia and normal erythrocytes which were stocked in an ice cold water bath for 4 weeks reacted with antihuman CRP serum. No reaction with this antiserum was observed during a week of storage under the same conditions. It is possible that an altered protein is formed during storage and that this reacts with antihuman CRP serum, since these changes occurred in all of the hemolysates.

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